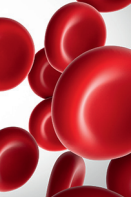




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TO THE EDITOR:

Premature aging of the immune system affects the response to SARS-CoV-2 mRNA vaccine in β -thalassemia: role of an additional dose

Rita Carsetti,^{1,*} Chiara Agrati,^{2,*} Valeria Maria Pinto,³ Barbara Giansin,^{3,4} Rita Gamberini,⁵ Monica Fortini,⁵ Susanna Barella,⁶ Rita Denotti,⁶ Silverio Perrotta,⁷ Maddalena Casale,⁷ Aurelio Maggio,⁸ Lorella Pitrolo,⁸ Eleonora Tartaglia,² Eva Piano Mortari,¹ Francesca Colavita,⁹ Vincenzo Puro,¹⁰ Massimo Francalancia,⁹ Valeria Marini,^{11,12} Marco Caminati,¹³ Filippo Mazzi,¹³ Lucia De Franceschi,¹³ Gian Luca Forni,³ and Franco Locatelli¹⁴

¹B-Cell Laboratory, Immunology Research Area, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Bambino Gesù Children’s Hospital, Rome, Italy; ²Laboratory of Cellular Immunology, IRCCS, INMI L. Spallanzani, Rome, Italy; ³Center for Microcythemia, Congenital Anemia, and Iron Dysmetabolism, Galliera Hospital, Genoa, Italy; ⁴ForAnemia Foundation, Genoa, Italy; ⁵Day Hospital della Talassemia e delle Emoglobinopatie, Dipartimento di Medicina, Azienda Ospedaliero-Universitaria S. Anna, Cona di Ferrara, Ferrara, Italy; ⁶SSD Talassemia, Ospedale Pediatrico Microcitemico “A. Cao”, ASL8, Cagliari, Italy; ⁷Dipartimento della Donna, del Bambino e di Chirurgia Generale e Specialistica, Università, degli Studi della Campania “Luigi Vanvitelli”, Napoli, Italy; ⁸Campus of Hematology Franco and Piera Cutino, AOOR Villa Sofia-V. Cervello, Palermo, Italy; ⁹Laboratory of Virology and ¹⁰Risk Management and Biosafety Unit, IRCCS, INMI L Spallanzani, Rome, Italy; ¹¹Clinical Pharmacology Unit, EO Ospedali Galliera, Genoa, Italy; ¹²Pharmacology & Toxicology Unit, Department of Internal Medicine, University of Genoa, Genoa, Italy; ¹³Department of Medicine, University of Verona & AOUI Verona, Policlinico GB Rossi, Verona, Italy; and ¹⁴Department of Pediatric Hematology and Oncology, IRCCS Bambino Gesù Children’s Hospital, Catholic University of the Sacred Heart, Rome, Italy

Patients with β -thalassemia show 5-fold increase in age-standardized lethality due to SARS-CoV-2 infection, representing a high-risk population compared with age- and sex-matched healthy subjects.¹ Vaccination against SARS-CoV-2 is crucial to reduce mortality and morbidity of frail patients.² Up to now, limited data have been available on the responses of patients with β -thalassemia immunized with anti-SARS-CoV-2 mRNA vaccines.³

The main aim of the present prospective multicenter study was to evaluate the immunogenicity of the SARS-CoV-2 mRNA vaccine BNT162b2 in patients with transfusion-dependent β -thalassemia (TDT). Patients with TDT (n = 154), vaccinated within the Nationwide Vaccination Program in Italy, were enrolled in 7 comprehensive Italian centers for hemoglobinopathies. Serum samples were collected before vaccination (T0), 2 weeks after the second dose (T1), 12 weeks after the second dose (T2), before the third dose (T3), and at 4 and 12 weeks after the third dose (respectively, T4 and T5) (supplemental Figure 1A, available on the *Blood* website). A group of healthcare workers (HCWs) enrolled at INMI L. Spallanzani served as control group. Previous and breakthrough SARS-CoV-2 infections were diagnosed by a positive molecular reverse transcriptase polymerase chain reaction test or seroconversion to antinucleocapsid antibodies. The response to vaccination was evaluated by quantifying anti-spike protein receptor-binding domain (RBD) antibodies (Abbott Laboratories, Wiesbaden, Germany). The spike protein-specific T-cell response was evaluated in a subset of patients (n = 12) by an interferon- γ -releasing assay performed on whole blood. In the same group of patients, spike protein-specific memory B cells were detected and quantified by flow cytometry or ELISpot. The study was

approved by the Comitato Etico dell’Istituto Nazionale per le Malattie, Infettive Lazzaro Spallanzani IRCCS as the National Review Committee Board for the COVID-19 pandemic in Italy, and registered with clinicaltrials.gov (NCT05157256). Overall, 154 patients with TDT and 82 HCWs were enrolled in this study; all subjects received the mRNA BNT162b2 vaccine. Demographic and clinical patients’ characteristics are summarized in [Table 1](#). After vaccinations, no serious adverse events related to vaccination were reported in the study population. Moreover, no COVID-related hospitalization or death was recorded in the study population.

As shown in [Figure 1A](#), after 2 doses (T1), infection-naïve patients TDT (n = 132) showed an optimal rate of seroconversion to anti-RBD IgG (131/132, 99.2%), similarly to the control HCWs (82/82, 100%). The anti-RBD titers of infection-naïve TDT and HCW subjects were similar at T1, thus confirming excellent response to the vaccine. At T2, however, 12 weeks after the second dose, anti-RBD antibodies were significantly lower in patients with TDT than in controls (estimated difference, 148 binding arbitrary units (BAU)/mL; confidence interval, 55-244 BAU/mL). At T3, just before the third dose, anti-RBD antibodies were lower in patients with TDT than in controls without reaching statistically significant difference (P = .056; estimated difference, 26.6 BAU/mL; confidence interval, -0.7 to 55 BAU/mL). Thus, notwithstanding the optimal response to the vaccine, antibody titers declined more rapidly in patients with TDT than in HCWs after the first 2 doses. Similar kinetics was observed for the spike protein-specific T- and B-cell responses ([Figure 1B](#); supplemental Figure 1B), confirming a good immune response early after vaccination that decreases faster over time in patients with TDT than in age-matched HCWs. The third dose was able to

Table 1. Main characteristics of subjects included in the analysis

Subjects' characteristics	Value*
HCWs	82
Men	21 (25.6%)
Women	61 (74.4%)
Age, y	45.0 (35.0-53.0)
SARS-CoV-2	
Naïve	82 (100%)
Previously infected	—
TDT patients	154
Men	61 (39.6%)
Women	93 (60.4%)
Age, y	44.0 (37.0-51.0)
Splenectomized	83 (53.9%)
ICT	154
DFX	80 (51.9%)
DFP	20 (13.0%)
DFO	18 (11.7%)
Combined	31 (20.1%)
Not available	5 (3.2%)
HU	17 (11.0%)
SARS-CoV-2	
Naïve	132 (85.7%)
Previously infected	22 (14.3%)

DFX, deferoxamine; DFP, deferiprone; DFX, deferasirox; HCWs, healthcare workers; HU, hydroxyurea; ICT, iron chelation therapy; TDT, transfusion-dependent β -thalassemia.

*Values are n, n (%), or median (interquartile range).

improve the immune response of patients with TDT (T4 vs T3, $P < .0001$), reaching 100% seropositivity rate, with a similar anti-RBD titer in the TDT and HCW groups (T4) (Figure 1B). Of note, the level of anti-RBD antibodies was higher at T4, 4 weeks after the third dose, than at T1 ($P < .0001$), demonstrating the beneficial effect of the third dose in achieving an optimal antibody titer in patients with TDT. Most importantly, at T5, 12 weeks after the third dose, antibody titers were higher than at T2 in both patients with TDT and HCWs, suggesting that the third vaccine dose may be able to generate an antibody production that is sustained for a longer time. We also evaluated the immune response of a small population of patients with TDT ($n = 22$) who received the BNT162b2 vaccine 1 to 3 months after SARS-CoV-2 infection. As shown in supplemental Figure 2A, previously infected patients with TDT showed significantly higher anti-RBD titer levels in response to vaccination at T1, T2, and T3 compared with infection-naïve patients with TDT. There was no difference at T4 and T5, corresponding respectively to 4 and 12 weeks after the booster dose (supplemental Figure 2A). Thus, we confirm the strength of hybrid immunity⁴ in patients with TDT, but we also

show that, after the booster dose, equally high antibody levels are produced in patients never infected by SARS-CoV-2.

We then assessed the possible impact of clinical variables on the humoral response. We first compared anti-RBD antibodies by age, sex, splenectomy, and chelation therapy. We found no evidence for any association at each time point except for age (Figure 1C; supplemental Figure 2). As shown in Figure 1C, we observed a slight negative correlation (Pearson correlation coefficient, R_p) between age and anti-RBD titer at T1 (T1: $R_p = -0.16$; $P = .068$), reaching statistical significance at T2, corresponding to 12 weeks after the second vaccine dose (T2: $R_p = -0.21$; $P = .019$). This correlation was lost at later time points and after the third vaccination. The behavior of anti-RBD antibody response to BNT162b2 vaccination in patients with TDT was similar to that reported in healthy subjects over 80 years of age,⁵⁻⁸ for whom the effect of age disappears after the third dose and further ones.⁵ Of note, evidence of premature aging of the immune system has been reported in patients with TDT.⁹⁻¹¹ This might be related to different factors, including multiple transfusions as alloantigen stimulation associated or iron overload, negatively affecting T_H response. Indeed, TDT patients have been shown to have (1) reduced CD4/CD8 ratio, (2) increased circulating interleukin (IL)-17 and transforming growth factor β with possible detrimental effect on T-cell immune response, and (3) reduction in IL-2 and interferon- γ production by activated lymphocytes from patients with TDT compared with healthy control subjects.¹²⁻¹⁴ A similar immunosenescent profile has also been invoked for the inverse relationship between neutralizing response and age in healthy elderly subjects, who are characterized by restriction of T- and B-cell repertoires^{15,16} and by general defects in CD4 and CD8 T-cell activation, differentiation, and function (proliferation and cytokines production), hindering a protective long-lasting immune response.¹⁷ Further analysis focusing on the expression of inhibitory/senescent markers on T cells could help in clarifying their role in dampening the strength as well as the persistence of vaccine-induced immunity both in naïve and in previously SARS-CoV-2-infected patients with TDT.

In conclusion, our data seem to indicate that immune response of patients with TDT to SARS-CoV-2 vaccination is similar to that reported in healthy elderly subjects, suggesting a premature aging of the immune system of patients with TDT. Our results demonstrate the relevance of a third dose of mRNA vaccine for patients with TDT, who represent a population at high risk of developing severe complications and fatal events related to SARS-CoV-2 infection. Antibodies, produced immediately after vaccination, are the product of short-lived plasmablasts, whereas maintenance of antibody concentrations over time depends on the function and number of long-lived plasma cells.¹⁸ The third vaccine dose is therefore needed to increase the number of long-lived plasma cells in patients with TDT, thus ensuring more effective protection, as shown for the elderly. It remains to be fully elucidated whether the third vaccine dose also improves the maintenance of memory T- and B-cell responses over time.

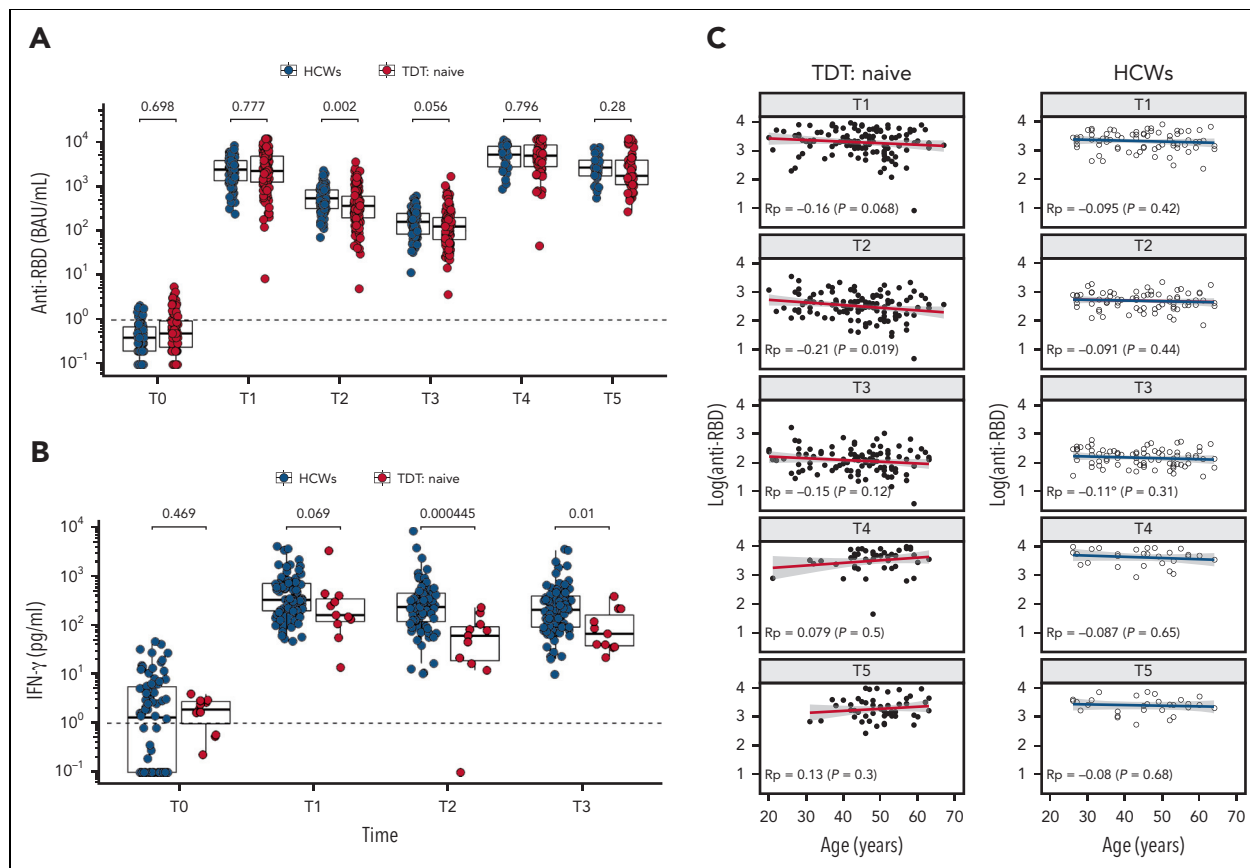


Figure 1. Kinetics of anti-RBD Abs and spike-specific T-cell response in patients with TDT compared with control subjects. (A) Comparison between health care workers (HCWs; open circles) and patients with transfusion-dependent β -thalassemia (TDT; red circles) naive to SARS-CoV-2 at each sampling time. (B) Spike-specific T cells (measured by interferon (IFN) γ release after specific stimulation) in the peripheral blood of control HCWs (open circles) and patients with TDT (red circles) measured at the indicated time points. (C) Correlation between anti-spike RBD antibodies and age in patients with TDT and healthy subjects (HCWs) naive to SARS-CoV-2. T0, before vaccination; T1, 2 weeks after the second dose; T2, 12 weeks after the second dose; T3, before the third dose; T4, 4 weeks after the third dose; T5, 12 weeks after the third dose; Rp, Pearson's correlation coefficient.

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Authorship

Contribution: G.L.F. and F.L. designed the research; R.G., M. Fortini, S.B., R.D., S.P., M. Casale, A.M., L.P., E.T., M. Caminati, F.M., and L.D.F. managed the patients; C.A., R.C., E.T., E.P.M., F.C., V.P., M. Francalancia, and V.M. performed research; R.C., C.A., and B.G. analyzed and interpreted data; B.G. performed statistical analysis; C.A., R.C., V.M.P., G.L.F., F.L., and L.D.F. wrote the manuscript; and all authors approved the final version of the manuscript.

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The current affiliation for C.A. is Department of Pediatric Hematology and Oncology, IRCCS Bambino Gesù Children's Hospital, Rome, Italy.

ORCID profiles: R.C., 0000-0002-2632-1078; V.M.P., 0000-0002-8375-6289; M. Fortini, 0000-0001-8406-0024; S.P., 0000-0001-6149-6845; E.T., 0000-0001-5773-7781; E.P.M., 0000-0002-0054-4732;

M. Francalancia, 0000-0003-3133-9237; M. Caminati, 0000-0001-7383-1487; L.D.F., 0000-0001-7093-777X; G.L.F., 0000-0001-9833-1016.

Correspondence: Gian Luca Forni, Centro della Microcitemia e delle Anemie, Congenite, Ospedale Galliera, Via Volta 6, Genova 16128, Italy; email: gianluca.forni@galliera.it.

Footnotes

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*R.C. and C.A. contributed equally to this work as first authors.

Data will be available on demand.

The online version of this article contains a data supplement.

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TO THE EDITOR:

Vaccine-induced immune thrombotic thrombocytopenia is mediated by a stereotyped clonotypic antibody

Jing Jing Wang,^{1,2} Bridie Armour,^{1,2} Tim Chataway,³ Alexander Troelnikov,^{1,2} Alex Colella,³ Olivia Yacoub,⁴ Simon Hockley,⁵ Chee Wee Tan,^{4,6} and Tom Paul Gordon^{1,2}

¹Department of Immunology, College of Medicine and Public Health, Flinders University, Bedford Park, SA, Australia; ²Department of Immunology, South Australia (SA) Pathology (Flinders Medical Centre), Bedford Park, SA, Australia; ³Flinders Proteomics Facility, College of Medicine and Public Health, Flinders University, Bedford Park, SA, Australia; ⁴SA Pathology, Adelaide, SA, Australia; ⁵Intensive Care Unit, Calvary Hospital, Adelaide, SA, Australia; and ⁶Department of Haematology, Royal Adelaide Hospital, Central Area Local Health Network (CALHN), Adelaide, SA, Australia

The syndrome of vaccine-induced immune thrombotic thrombocytopenia (VITT) is a rare thromboembolic complication of adenoviral-vectored severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines ChAdOx1 nCoV-19 (AstraZeneca) and Ad26.COV2.S (Janssen/Johnson & Johnson) mediated by antibodies directed against platelet factor 4 (PF4).¹⁻⁵ The mechanisms by which the adenoviral DNA vectors break immune tolerance to PF4 and trigger B-cell clonal expansion and secretion of anti-PF4 immunoglobulin Gs (IgGs) are under intense investigation and likely involve formation of immunogenic complexes of PF4 with vaccine components in a proinflammatory setting.⁶ Pathogenic anti-PF4 IgGs subsequently form circulating immune complexes with PF4 tetramers, which are thought to drive thrombotic events by Fc γ receptor IIa-dependent platelet activation and to activate granulocytes to

release procoagulant neutrophil extracellular traps.⁶⁻⁸ Serum anti-PF4 antibodies are mostly transient and appear in serum within days of vaccination, suggesting a recall immune response on memory B cells.⁹

Given their causal role in VITT, identification of the molecular composition of the anti-PF4 antibodies and their antigenic target(s) is crucial for better understanding of the pathogenesis and for developing better diagnostics and treatments. In a key advance, Huynh et al have mapped the antibody-binding site to a single conformational epitope on the PF4 molecule, which is located within the heparin-binding site and distinct from epitopes bound by serum from patients with heparin-induced thrombocytopenia (HIT).¹⁰ Moreover, a recent intact mass spectrometric analysis of anti-PF4 IgGs in patients with VITT and HIT revealed